MEMO
To: Lee Lynd, Bruce Dale, Sandy McLaughlin, Jim Kiniry, Michael Casler, Hans Jung
From: Mark Laser
Cc: Eric Larson, Haiming Jin, Fuat Celik, Kemantha Jayawardhana, Nathanael Greene, John Sheehan

Subject: Revised methodology for developing model switchgrass compositions
Date: February 2, 2004

I. Background

As discussed in an earlier memo (12/18/03; see below), the Role of Biomass in America's Energy Future project analysis requires that we use justifiable feedstock composition values that can withstand critical inspection. The memo presented a methodology to develop model compositions, the basis of which was discussed during a 1/8/04 conference call. Several points were raised during the call suggesting that the methodology be revised to:

- Distinguish between two harvesting schemes: 1) a single-cut, late season harvest; and 2) a 2-cut scheme with 1 early season harvest, and 1 late season harvest.

- More accurately account for maturation effects: both cell wall carbohydrate (cellulose and hemicellulose) and lignin increase with maturity; protein and soluble carbohydrates decrease with maturity.

- Differentiate between cell wall carbohydrate and soluble carbohydrate.

II. Revised methodology

A. Reference case

I've revised the methodology to address the above concerns, paying particular attention to adequately characterizing maturation effects. The NREL switchgrass composition database (DOE, 2003) remains the starting point. The database contains data for single-cut harvests from two different years: 14 samples from October 1, 1991 and 25 from August 1, 1992. I've disregarded the October harvest, however, as it had been grazed by beef steers earlier in the year (Wiselogel et al., 1996). A reference case (Table 1) was calculated from the set of August samples as follows:

1) Begin with normalized average from NREL database for August 1 harvest date (Table 1, Column B).

2) Explicitly account for extractives using data from Thammasouk et al. (Table 2).

3) Add acetate using mass fraction from Thammasouk et al. (0.09*xylan; Table 1, Column D).

4) Normalize composition (Table 1, Column E).

B. Component maturation rates

The following linear rates of change as a function of maturity are used (see Table 3):

1) Cell wall carbohydrate

   The reference case is assumed to be at a heading stage of maturity. The linear rate of change in cell wall carbohydrate (quantified as neutral detergent fiber – acid dissolved lignin) as a function of day of the year for vegetative to heading stages of growth—9.01×10^{-4} mass fraction cell wall carbohydrate/day—represents the average of that calculated from Jung and Vogel, 1992 (assuming 0.54 leaf to stem ratio calculated from Lemus et al., 2002) and Burns et al., 1997 (Figure 1). For post heading changes, the average linear rate reported by Casler and Boe, 2003 is used: 4.84×10^{-4} mass fraction cell wall carbohydrate/day.

2) Lignin

   The linear rate of change in lignin as a function of day of the year for vegetative to heading stages of growth—6.12×10^{-4} mass fraction lignin/day—represents the average of that calculated from Jung and Vogel, 1992 (assuming 0.54 leaf to stem ratio calculated from Lemus et al., 2002) and Burns et al., 1997 (Figure 2). For post heading changes, the average linear rate reported by Casler and Boe, 2003 is used: 2.82×10^{-4} mass fraction cell lignin/day.

C. Model cases

The following model cases are developed from the reference case as follows (compositions are summarized in Table 4; harvest dates are shown in Figure 3):

1) Base case, 2-cut, spring harvest

   - Start with reference composition
   - Assume mid-June harvest date: 45 days before reference harvest
   - Cell wall carbohydrate mass fraction decreases relative to reference case according to rate for vegetative to heading growth stages (Table 3).
   - Lignin mass fraction decreases relative to reference case according to rate for vegetative to heading growth stages (Table 3).
   - Protein mass fraction assumed to be 0.10, based on Brejda et al., 1994, Burns et al., 1997, and B. Dale, personal communication, for early season harvests.

- Ash fraction remains same as reference case.
- Acetate/xylan ratio is assumed to remain same as reference case at 0.0903. (Acetate is a component of hemicellulose.)
- Uronic acid/xylan ratio is assumed to remain same as reference case at 0.0865. (Uronic acid is a component of hemicellulose.)
- Remaining mass is divided among soluble carbohydrates according to reference case proportions.

2) Base-case, 2-cut, fall harvest

- Assume November 1 harvest date.
- Assume period from June 15 cut to heading is 90 days (Sept. 15); therefore, second harvest comes 45 days after heading
- Cell wall carbohydrate mass fraction increases relative to reference case according to rate for post heading (Table 3).
- Lignin carbohydrate mass fraction increases relative to reference case according to rate for post heading (Table 3).
- Acetate/xylan ratio is assumed to remain constant at 0.0903.
- Uronic acid/xylan ratio is assumed to remain constant at 0.0865.
- Remaining mass is divided among soluble carbohydrates and protein according to reference case proportions.

3) Base-case, 1-cut fall harvest

- Assume November 1 harvest date, or 90 days after heading
- Cell wall carbohydrate mass fraction increases relative to reference case according to rate for post heading (Table 3).
- Lignin carbohydrate mass fraction increases relative to reference case according to rate for post heading (Table 3).
- Acetate/xylan ratio is assumed to remain constant at 0.0903.
- Uronic acid/xylan ratio is assumed to remain constant at 0.0865.

- Remaining mass is divided among soluble carbohydrates according reference case proportions.

4) Advanced cases

- Assume cell wall carbohydrate increases 10% relative to corresponding base case harvest

- Assume ash is reduced by 50% relative to reference case.

- Acetate/xylan ratio is assumed to remain constant at 0.0903.

- Uronic acid/xylan ratio is assumed to remain constant at 0.0865.

- Remaining mass is divided among lignin, soluble carbohydrate, and protein using corresponding base case harvest.

Table 1. Reference case composition determination.

Component	(A) NREL Database Average	(B) Normalized Average	(C) Extractives Fraction	(D) Average + Acetate	(E) Reference Case
Cellulose	0.3345	0.3229		0.3229	0.3168
Hemicellulose	0.2651	0.2560		0.2560	0.2511
Soluble carbohydrate		0.0000	0.0307	0.0307	0.0301
Glucan	0.3345	0.3229		0.3229	0.3168
Glucan (soluble)			0.0242	0.0242	0.0238
Xylan	0.2222	0.2145		0.2145	0.2105
Arabinan	0.0299	0.0289		0.0289	0.0283
Arabinan (soluble)			0.0007	0.0007	0.0007
Galactan	0.0099	0.0096		0.0096	0.0094
Galactan (soluble)			0.0058	0.0058	0.0057
Mannan	0.0030	0.0029		0.0029	0.0029
Total carbohydrate		0.0000		0.6095	0.5979
Uronic acid	0.0192	0.0185		0.0185	0.0182
Total lignin	0.1813	0.1751	0.0656	0.2406	0.2361
Protein	0.0530	0.0511	0.0009	0.0520	0.0510
Extractives	0.1192	0.1151		0.1151	
Ash	0.0635	0.0613	0.0180	0.0793	0.0778
Acetate				0.0190	0.0190
Mass Closure	1.0358	1.0000	0.1151	1.0190	1.0000

Table 2. Composition of ethanol extractives from switchgrass (Thammasouk et al., 1997).

Component	Mass Fraction (dry wt. basis)	Normalized Mass Fraction
Total carbohydrate	0.2070	0.2663
Glucan	0.1635	0.2103
Xylan	b.d.l.	-
Galactan	0.0390	0.0502
Arabinan	0.0045	0.0058
Mannan	b.d.l.	-
Total lignin	0.4428	0.5697
Klason lignin	0.4067	0.5232
Acid soluble lignin	0.0361	0.0464
Ash	0.1215	0.1563
Protein	0.0060	0.0077
Total	0.7773	1.0000

b.d.l. = “below detection limit”

Table 3. Model cell wall component linear maturation rates.

Maturation Effect	Vegetative-to-heading (mass fraction/day) [a]	Post heading (mass fraction/day) [b]
Cell wall carbohydrate	9.01E-04	4.84E-04
Lignin	6.12E-04	2.82E-04

[a]Average calculated from Jung and Vogel, 1992 (assuming 0.54 leaf to stem ratio calculated from Lemus et al., 2002) and Burns et al., 1997.
[b]Average from Casler and Boe, 2003.

Table 4. Model switchgrass compositions.

Component	Base Case 1-cut (Late Fall)	Base Case 2-cut (Spring)	Base Case 2-cut (Fall)	Advanced Case 1-cut (Late Fall)	Advanced Case 2-cut (Spring)	Advanced Case 2-cut (Fall)
Cellulose	0.3410	0.2942	0.3289	0.3751	0.3236	0.3618
Hemicellulose	0.2703	0.2341	0.2607	0.2974	0.2575	0.2868
Soluble carbohydrate	0.0035	0.0508	0.0168	0.0032	0.0484	0.0154
Glucan	0.3410	0.2942	0.3289	0.3751	0.3236	0.3618
Glucan (soluble)	0.0028	0.0401	0.0133	0.0025	0.0382	0.0122
Xylan	0.2266	0.1962	0.2185	0.2493	0.2158	0.2404
Arabinan	0.0305	0.0264	0.0294	0.0335	0.0290	0.0323
Arabinan (soluble)	0.0001	0.0011	0.0004	0.0001	0.0011	0.0003
Galactan	0.0101	0.0088	0.0098	0.0112	0.0097	0.0108
Galactan (soluble)	0.0007	0.0096	0.0032	0.0006	0.0091	0.0029
Mannan	0.0031	0.0027	0.0030	0.0034	0.0029	0.0033
Total carbohydrate	0.6149	0.5790	0.6064	0.6757	0.6294	0.6640
Uronic acid	0.0196	0.0170	0.0189	0.0215	0.0186	0.0208
Total lignin	0.2614	0.2085	0.2487	0.2361	0.1984	0.2285
Protein	0.0059	0.1000	0.0285	0.0054	0.0952	0.0262
Extractives						
Ash	0.0778	0.0778	0.0778	0.0389	0.0389	0.0389
Acetate	0.0205	0.0177	0.0197	0.0225	0.0195	0.0217
Mass Closure	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
LHV	7194	7052	7160	7378	7285	7360
HHV	7798	7654	7763	8001	7906	7981
Elemental Composition						
C	0.4966	0.4792	0.4925	0.5056	0.4932	0.5031
H	0.0644	0.0641	0.0643	0.0661	0.0662	0.0661
O	0.3603	0.3649	0.3614	0.3887	0.3884	0.3883
N	0.0008	0.0136	0.0039	0.0007	0.0130	0.0036
S	0.0000	0.0004	0.0001	0.0000	0.0004	0.0001
Ash	0.0778	0.0778	0.0778	0.0389	0.0389	0.0389
TOTAL	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

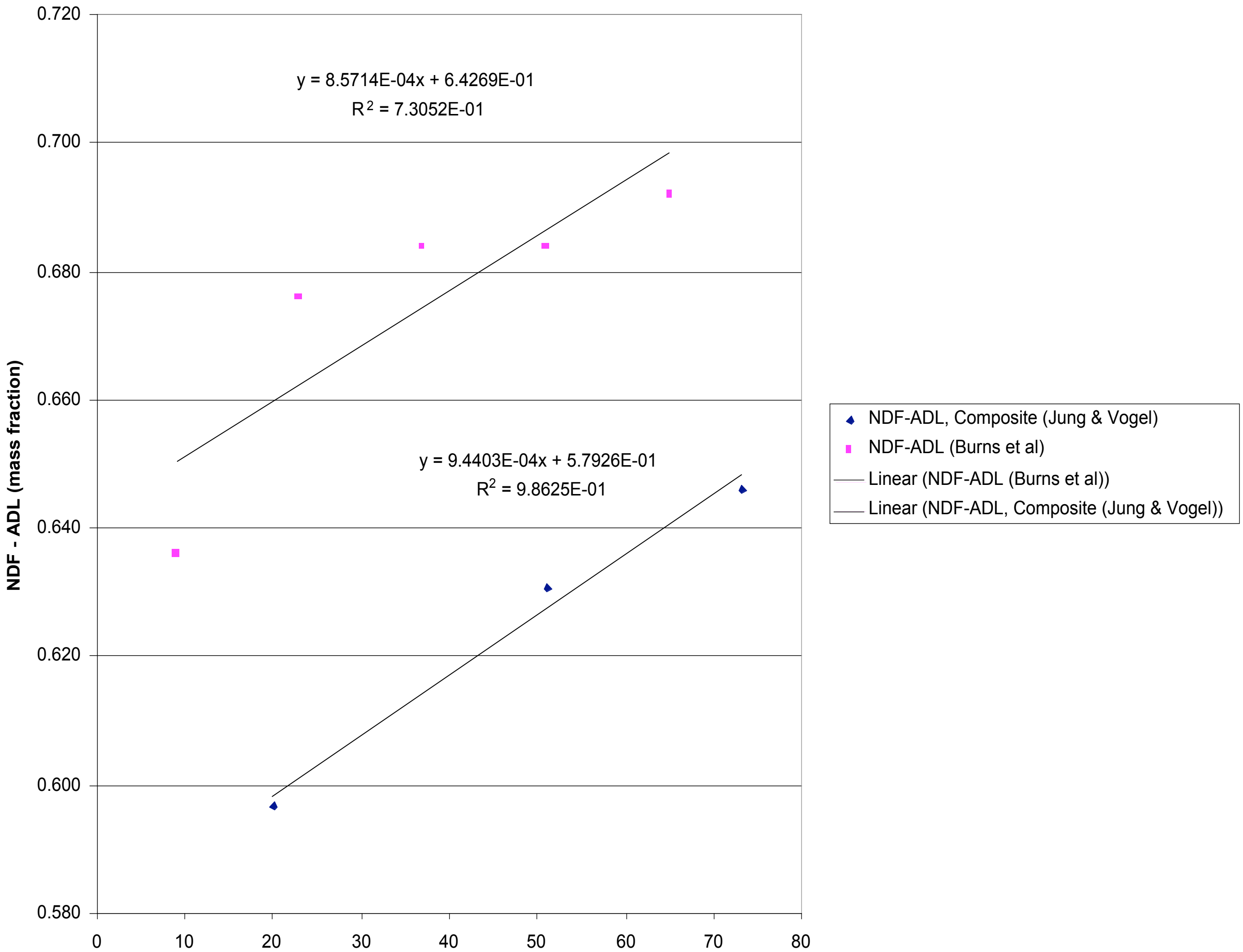

Figure 1. Switchgrass cell wall carbohydrate (NDF-ADL) as a function of maturity linear regressions.

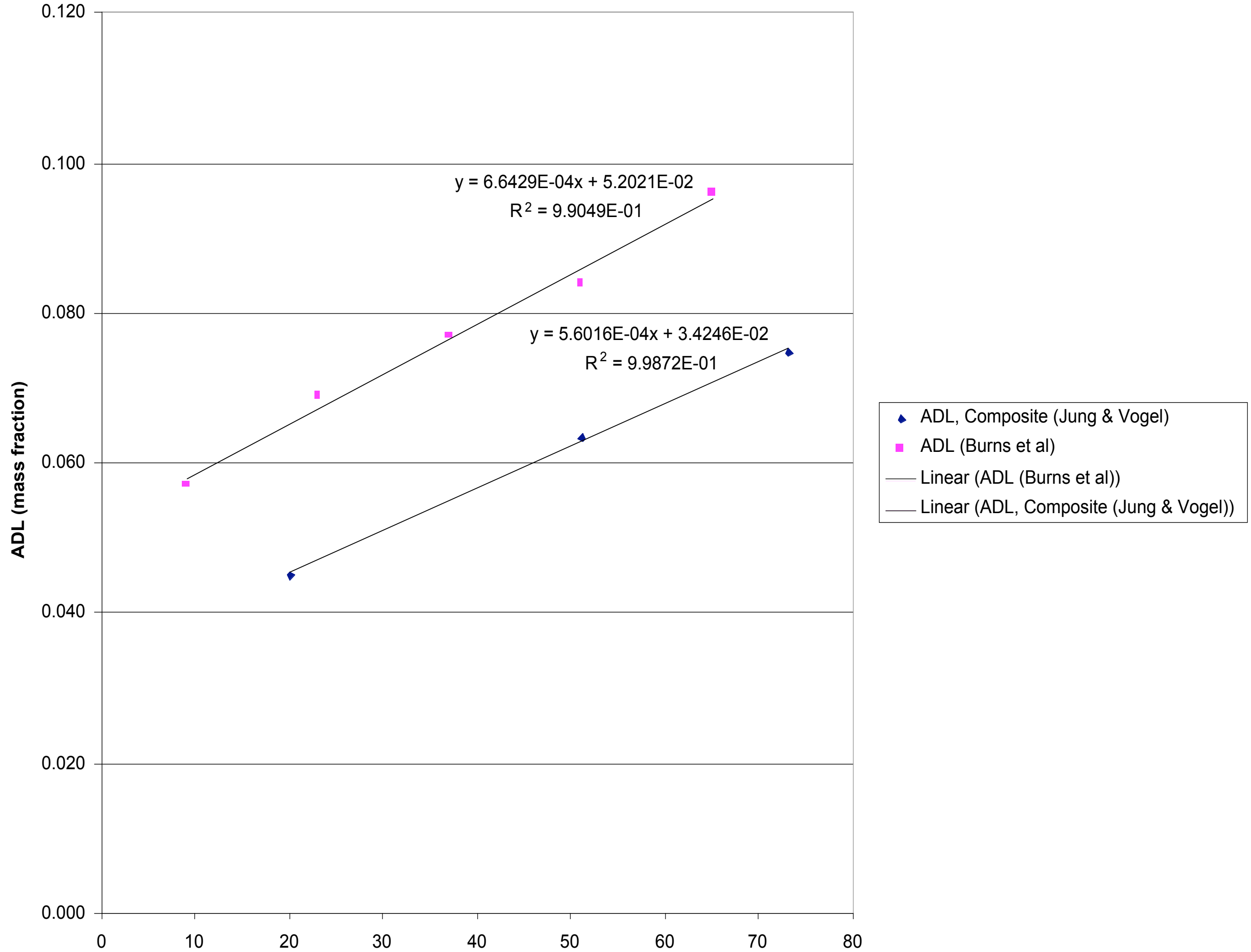

Figure 2. Switchgrass lignin (ADL) as a function of maturity linear regressions.

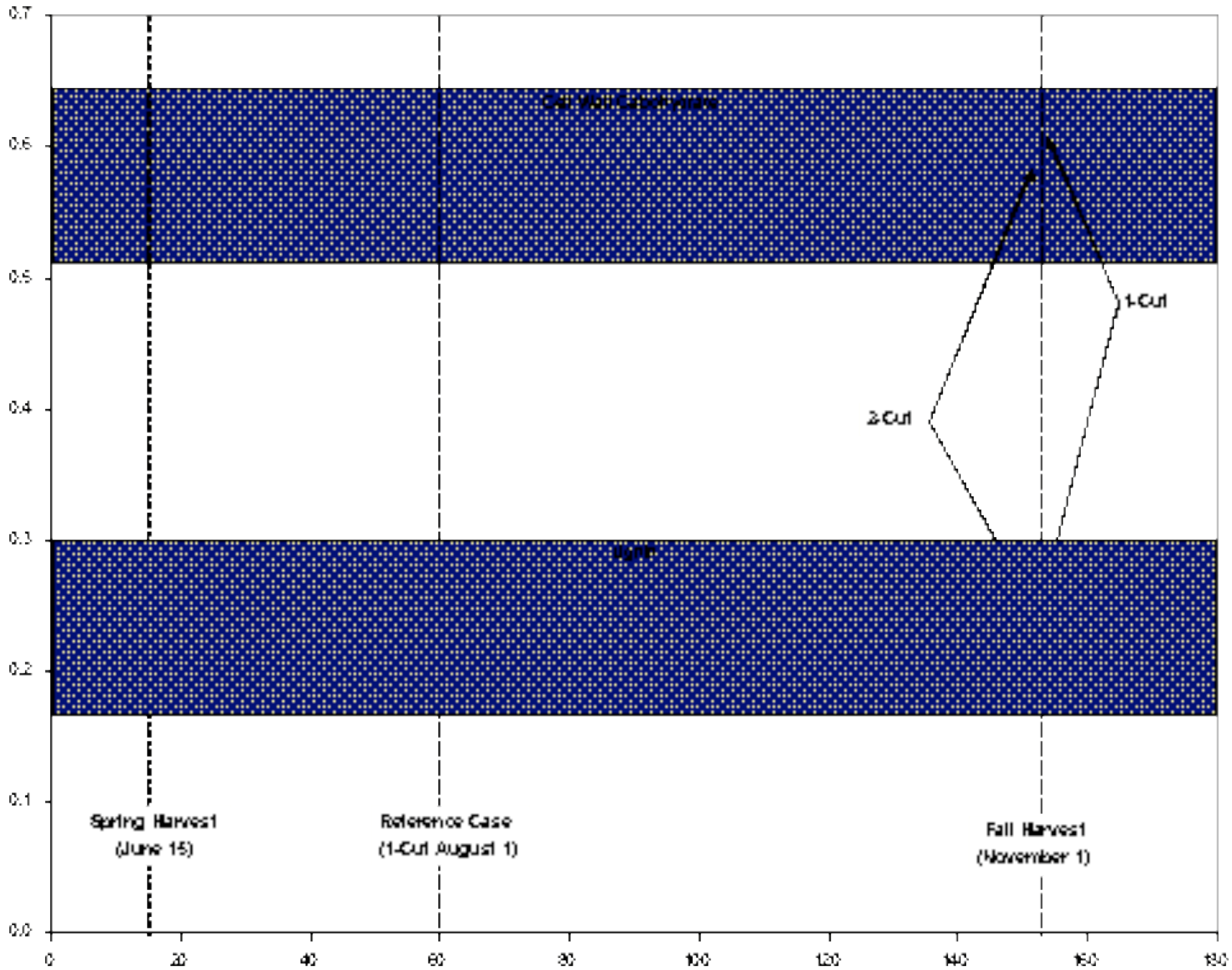

Figure 3. Harvesting dates and cell wall composition for base cases and reference case.

D. Literature cited

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MEMO
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Subject: Switchgrass composition determination
Date: December 18, 2003

I. Executive Summary

Given that the RBAEF technical analysis starts with and greatly depends upon the feedstock composition, it's essential that we use justifiable values that can withstand critical inspection. This memo addresses this concern by:

1) Providing a review of switchgrass compositions reported in the literature and of analytical methods used to determine composition. Eleven reports of switchgrass composition are reviewed (Table 1); acid hydrolysis and detergent fiber compositional analysis methods are summarized.

2) Proposing base-case (Table 5) and advanced-case compositions for first- and second-cut harvests using the literature review as a guide (Table 7). Two advanced case scenarios are included: 1) low ash/high carbohydrate (1^{st} and 2^{nd} cut harvests); and 2) low ash/high lignin (1^{st} and 2^{nd} cut harvests).

3) Facilitating a discussion among the group with the intended outcome of establishing and agreeing upon defensible switchgrass compositions for use in our analysis.

The memo is intended to serve as reference material for an anticipated conference call involving members of the RBAEF group as well as Michael Casler, an expert in plant breeding from the University of Wisconsin, and Hans Jung, a USDA-ARS agronomist from the University of Minnesota with expertise in forage cell wall structure and digestibility. Here are some talking points and questions that we've identified thus far:

- Is the methodology for estimating composition sound? Is there a better alternative?

- Will a 2-cut harvesting scheme compromise long-term switchgrass productivity? Will it require more fertilizer or that the crop be replanted more frequently?

- Given an aggressive plant breeding program carried out over at least 20 years, by how much is it reasonable to expect that ash content can be decreased without significantly compromising productivity?

- By how much is it reasonable to expect the carbohydrate:lignin ratio to be changed (up or down) in a low-ash switchgrass without significantly compromising productivity?

- Review and agree on a composition for: 1) base case (1st & 2nd cut); 2) advanced case A (low ash/high carbohydrate; 1st & 2nd cut); 3) advanced case B (low ash/high lignin; 1st & 2nd cut).

To facilitate discussion of the last point, Table 8 summarizes the proposed base case and advanced case scenarios and provides space for you to indicate whether the proposed values are appropriate.

II. Switchgrass composition references

As can be seen from Table 1, which lists switchgrass composition values from 11 references in the literature, significant variability exists among reported data, especially for cellulose (29.8% - 44.9%), hemicellulose (24.4% - 35.1%), total carbohydrate (51.2% to 81%), and lignin (5.5% - 24.8%). Some of this variability likely results from the different assays used to quantify these components, summarized as follows:

Carbohydrate analysis. Two methods are commonly used:

1. 2-stage acid hydrolysis (AH), in which cellulose and hemicellulose are hydrolyzed with strong acid and resulting monomeric sugars quantified with GC or HPLC

2. Forage fiber analysis (FFA), which measures:

   a. Acid detergent fiber (ADF)—an acidified detergent solution is used to dissolve cell solubles, hemicellulose and soluble minerals, leaving a residue of cellulose, lignin, heat damaged protein, and a portion of cell wall protein, and minerals (ash).

   b. Neutral detergent fiber (NDF)—a neutral detergent solution (sodium sulfite) is used to dissolve the easily digested pectins and plant cell contents (proteins, sugars, and lipids), leaving a fibrous residue that is primarily cell wall components of plants, including cellulose, hemicellulose, and lignin.

   c. Acid detergent lignin (ADL)—a strong acid solution is used to hydrolyze ADF cellulose, hemicellulose, and protein, leaving lignin and acid insoluble ash (AIA).

   d. Acid insoluble ash (AIA)—a strong acid is used to dissolve soluble portion of total ash sample (see below), leaving acid insoluble portion.

   e. Total ash—biomass sample is ignited in furnace, removing all organic material.

   Cellulose = ADF – ADL
   Hemicellulose = NDF – ADF
   Lignin = ADL – AIA

Acid hydrolysis provides a more direct measurement and allows characterization of specific sugars. Cellulose concentrations as measured by FFA are overestimated when xylans are present in ADF and underestimated when heat-damaged protein is present in ADL. Hemicellulose concentrations measured by FFA are overestimated when protein is present in NDF and underestimated when residual xylan remains in ADF (12). Wiselogel et al. (1)—who employed acid hydrolysis—compared their results with those of Sladden et al. (10) who used FFA and found that cellulose content was similar for the two studies, but that hemicellulose was lower (25% vs. 30%) for their acid hydrolysis method (see columns 1 and 10, Table 1). It's important to note, however, that Wiselogel et al. performed an extractives analysis *before* analyzing the remaining solids for carbohydrate content, *without* determining the composition of the extracted material. It's very likely that their extractives fraction contained sugars (in fact, they explicitly state that extractives typically contain water soluble carbohydrates) that might have accounted for their hemicellulose content being lower than that of Sladden et al. Thammasouk et al (11) examined the composition of switchgrass extractives (Table 2) and found that the extractives contained 20.7% carbohydrate, 44% lignin, 12% ash, and 0.6% protein.

Lignin analysis. Three methods are commonly used:

1. Klason lignin (KL), in which acid hydrolysis is used to remove cellulose, hemicellulose, and protein, leaving a lignin residue.

2. Acid detergent lignin (ADL), as described above.

3. Permanganate lignin (PL), in which a potassium manganate solution is used to dissolve ADF lignin, leaving a cellulose rich residue: fraction lignin = (ADF sample – PL residue)/ADF sample.

The Klason lignin method is considered to more accurate than either ADL or PL, which both tend to underestimate lignin (by about 2 to 5 times) due to dissolution of lignin during the ADF portion of the analyses (5, 12).

III. Review of the reported data

In examining the data, it's useful to keep in mind our common sense criteria:

- Accurate determination of cellulose, hemicellulose (preferably with quantification of individual hemicellulose sugars), lignin, and protein mass fraction (dry weight basis).
- High mass balance closure, ideally $\geq$ 95%.
- Representative compositions for both first-cut and second-cut harvests.

Four studies used FFA (3, 4, 7, 10) which measures cellulose and hemicellulose by difference as opposed to directly and does not quantify individual hemicellulose sugars. Of the six references that employed acid hydrolysis, two did not differentiate between cellulose and hemicellulose (5, 6); two did not determine the composition of sizable extractive fractions (1, 2); and two had poor mass balance closure (5, 8). Four used either ADL or PL lignin analysis (3, 4, 7, 10) which underestimates lignin content, and two did not report protein concentration (6, 8). The reference that used the most accurate methodology across the board—acid hydrolysis without preliminary

extraction for carbohydrate analysis and Klason lignin analysis—(column 11a) did not report information about harvest time (though, from the high lignin/low protein composition, the sample likely came from a fall harvest). Furthermore, the study only considered one switchgrass sample. Only two studies reported data for both first and second cuts (3/4 and 10); both used FFA carbohydrate analysis and ADL lignin analysis. Table 3 summarizes the advantages and disadvantages of each reference.

IV. Developing representative compositions

The NREL database (2), in our opinion, emerges as the best reference from which to construct a base-case composition: it is the most comprehensive—containing 39 sample—and employs the most accurate methodology for measuring carbohydrate and lignin, except that neither the composition of the extractives fraction nor the amount of acetate contained in the samples was determined. We can, however, compensate for these limitations by using the extractives composition and acetate fraction reported by Thammasouk et al. (11, Table 2) who analyzed a sample obtained from NREL. Beginning with average, normalized values from the NREL database (column B), we use the normalized extractives composition listed in Table 2 to estimate the amount of each component contained in the extractives fraction (column C):

$$m_{ex,\,i} = f_{ex} * x_{ex,\,i} \qquad (1)$$

where:

$m_{ex,\,i}$ = mass of ith component in extractives fraction (column C, Table 4)
f_{ex} = mass fraction extractives (column B, Table 4)
$x_{ex,\,i}$ = mass fraction of ith component in extractives (from Table 2)

(As an example, the glucan contained in the extractives is calculated as 0.1134*0.2103 = 0.0239.) Columns B and C in Table 4 are then added to yield an overall composition that accounts for components contained in the extractives (column D). To account for acetate, which is not reported in the NREL database but is important to consider due to its impact on biological processing (e.g. inhibitory effects, reaction with ammonia in AFEX pretreatment, etc.), we use the mass fraction reported by Thammasouk et al. (11)—0.0190—and reduce the mass fractions of the other components proportionally as follows:

$$f_{i\ w/acetate} = f_{i\ w/o\ acetate} * (1 - f_{acetate}) \qquad (2)$$

where:

$f_{i\ w/acetate}$ = fraction of ith component in switchgrass, including acetate (column E, Table 4)
$f_{i\ w/o\ acetate}$ = fraction of ith component in switchgrass without acetate (column D, Table 4)
$f_{acetate}$ = fraction acetate (column E, Table 4)

(As an example, the fraction glucan contained in switchgrass + acetate sample is calculated as 0.3494*(1-0.0190) = 0.3427.) This results in the base-case composition listed in column E. It should be noted that the samples in the NREL database upon which this base case is based are from *late-season, single-cut harvests*. We must therefore make several assumptions in extending this to establish representative compositions for early season first-cut and late-season second-cut

harvests for both near-term/base case and long-term/advanced case scenarios. We assume the following:

1. Late-season, second-cut harvest composition is equal to the base case composition presented in column E, Table 4. Late-season, second-cut switchgrass harvests have been shown to be higher in protein and lower in neutral detergent fiber than late-season single-cut harvests (20). We nonetheless assume the two harvests have equal composition for the sake of simplicity, because the protein fraction computed in Table 4 (4.8%) is well within the range reported by Sanderson et al (20) for late-season, second-cut switchgrass (3.0 – 6.1%), because the reported increase in NDF was slight (2 – 3%), and because explicit changes in cellulose, hemicellulose, and lignin as a function of harvest frequency were not evaluated—NDF is a composite measure of these components.

2. The early season, first-cut harvest protein fraction is assumed to be 0.10 based on Brejda et al., 1994 (ref. 14), Burns et al., 1997 (ref. 15), and B. Dale, personal communication. Early season, first-cut harvests are typically higher in protein (19, 20) and lower in lignin (21) relative to late-season harvests.

3. The carbohydrate fraction is held constant across harvests. Early season harvests have been shown to be lower in cellulose relative to late-season single-cut harvests (22) and lower in NDF (20). Forage grasses, however, are typically higher in soluble carbohydrate (26, 27) relative to late-season harvests. We were unable to find studies comparing total carbohydrate content of early season harvests and late-season second-cut harvests and thus have kept carbohydrate content constant.

4. The ash fraction is held constant across harvests. Early season switchgrass harvests are generally higher in ash relative to late season single-cut harvests (22, 23). We were unable, however, to find studies comparing ash composition of early season harvests and late-season second-cut harvests and thus have held ash content constant.

5. The early season, first-cut lignin content is reduced to offset the increase in protein relative to the late-season, second-cut.

The resulting base case, first- and second-cut compositions are listed in Table 5. Table 5 also lists the calculated elemental composition of each harvest using the molecular formulas indicated.

It's important to keep in mind the RBAEF project's focus upon mature technology, including mature switchgrass production technology and the potential of crop breeding and selection to alter composition (for a review of this concept, see reference 13). In Table 7, we propose compositions for two advanced case scenarios: 1) switchgrass having an elevated carbohydrate content for use as a bioethanol feedstock; and 2) having an elevated energy density for use as a biopower feedstock. The assumptions we used to create these scenarios are as follows:

- We reduced the ash content by 50% from 7.6% to 3.79% for both scenarios, and increased the values of all other components proportionately. This ash content is at the

lower end of the range presented in Table 1. The reduction is consistent with the assertion made by Casler and Boe (25) that it should be possible to lower ash content through selection and breeding.

- For the enhanced carbohydrate scenario, we then increased the carbohydrate fraction by 10% and reduced all other components—except protein and ash—proportionately.

- For the enhanced lignin scenario, we increased the lignin fraction by 10% and reduced all other components—except protein and ash—proportionately.

Such changes in carbohydrate and lignin are considered feasible (greater changes may indeed be possible, though ultimate limits are unknown at present) in a mature technology timeframe (e.g. 20 years) by experts in plant breeding (Michael Casler, personal communication). Comparable changes have already been realized in forage crops with the goal of improving quality for ruminant livestock—Casler and Vogel (24), for example, cite gains in in vitro dry matter digestibility (IVDMD) as high as 11.8% per cycle. It's also worth noting that the advanced case, enhanced carbohydrate content—69%—is within the range of literature values reported in Table 1 for acid hydrolysis-based assays (51% - 81%), suggesting that it represents an achievable target.

Table 1. Comparison of switchgrass composition values reported in the literature (dry weight basis).

Reference	1	2	3	4	5	6[c]	7	8	9	10a	10b	11a	11b
Metric													
Cellulose	0.332	0.333	0.407	0.449	n.r.	n.r.	0.371	0.322	0.440	0.310	0.298	0.313	0.299
Hemicellulose	0.252	0.264	0.351	0.314	n.r.	n.r.	0.321	0.244	0.241	0.310	0.302	0.256	0.246
Glucan	0.332	0.333	n.r.	n.r.	n.r.	n.r.	n.r.	0.322	0.445	n.r.	n.r.	0.313	0.299
Xylan	0.213	0.222	n.r.	n.r.	n.r.	n.r.	n.r.	0.203	0.215	n.r.	n.r.	0.206	0.199
Arabinan	0.028	0.029	n.r.	n.r.	n.r.	n.r.	n.r.	0.037	0.026	n.r.	n.r.	0.031	0.034
Galactan	0.009	0.010	n.r.	n.r.	n.r.	n.r.	n.r.	b.d.l.	n.r.	n.r.	n.r.	0.019	0.014
Mannan	0.003	0.003	n.r.	n.r.	n.r.	n.r.	n.r.	0.004	n.r.	n.r.	n.r.	0.031	0.034
Starch	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	0.005	n.r.	n.r.	n.r.	n.r.
Holocellulose	n.r.	n.r.	n.r.	n.r.	n.r.	0.810	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Total carbohydrate	0.584	0.597	0.758	0.753	0.512	0.810	0.692	0.566	0.686	0.620	0.600	0.569	0.545
Sugars method	AH	AH	FFA	FFA	AH	AH	FFA	AH	unknown	FFA	FFA	AH	AH
Lipid	n.r.	n.r.	n.r.	n.r.	0.013	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Acetate	0.015[d]	n.r.	n.r.	n.r.	0.014	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	0.019	0.018
Lactate	n.r.	n.r.	n.r.	n.r.	b.d.l.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Malate	n.r.	n.r.	n.r.	n.r.	0.010	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Uronic acid	0.015[d]	0.014	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	0.019	0.015
Lignin	0.147	0.186	0.055	0.120	0.142	0.195	0.063	0.195	0.205	0.072	0.072	0.214	0.179
Acid insoluble lignin	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	0.037	n.r.	n.r.	n.r.	0.034	0.024
Total lignin	0.147	0.186	0.055	0.120	0.142	0.195	0.063	0.232	0.205	0.072	0.072	0.248	0.201
Lignin method	KL	KL	ADL	ADL	KL	KL	ADL	KL	unknown	PL	PL	KL	KL
Protein	0.030	0.049	0.098[a]	0.040	0.093	n.r.	0.034	n.r.	0.019	0.072	0.072	0.039	0.037
Protein method	6.25*N	6.25*N					6.25*N		unknown	6.25*N	6.25*N	6.25*N	6.25*N
Extractives	0.156	0.116	n.r.	n.r.	n.r.	0.12 - 0.20	n.r.	n.r.	n.r.	n.r.	n.r.	n.d.	0.097
Extractives method	95% etoh	95% etoh				Hot water							
Ash	0.053	0.061	0.058	0.046	0.083	0.041	0.062	0.071	0.035	0.072	0.089	0.071	0.057
Other	n.r.	n.r.	0.031	0.031	n.r.	n.r.	n.r.	0.120	0.055	n.r.	n.r.	n.r.	n.r.
Mass Closure	1.000	1.023	0.969	0.969	0.867	1.046	0.871	0.880	0.945	0.836	0.834	0.964	0.971
Harvest time	Fall	Fall	Spring	Fall	n.r.	Over-wintered Spring	Fall	n.r.	unknown	First cut	Second cut	n.r.	n.r.
No. samples	2	39	1	1	1	1	20	1	unknown	8	8	1	1

AH = acid hydrolysis; FFA = forage fiber analysis; KL = Klason lignin; ADL = acid detergent lignin; PL = permanganate lignin

n.r.: "not reported"

n.d.: "not determined"

b.d.l. = "below detection limit"

[a]Estimated by taking difference of 12.9 reported as "other" and 3.1 reported as "other" in reference 3.

[c]Values are for plant stem.

[d]Reported together: acetate + uronic acid = 3%.

Table 2. Composition of ethanol extractives from switchgrass (source: 11).

Component	Mass Fraction (dry wt. basis)	Normalized Mass Fraction
Total carbohydrate	0.2070	0.2663
Glucan	0.1635	0.2103
Xylan	b.d.l.	-
Galactan	0.0390	0.0502
Arabinan	0.0045	0.0058
Mannan	b.d.l.	-
Total lignin	0.4428	0.5697
Klason lignin	0.4067	0.5232
Acid soluble lignin	0.0361	0.0464
Ash	0.1215	0.1563
Protein	0.0060	0.0077
Total	0.7773	1.0000

b.d.l. = "below detection limit"

Table 3. Advantages and disadvantages of studies considered.

Reference	**Advantages**	**Disadvantages**
1	▪ Acid hydrolysis method for carbohydrates ▪ Klason method for lignin ▪ Good mass balance closure	▪ Composition of extractives not determined ▪ Only one sample considered
2	▪ Acid hydrolysis method for carbohydrates ▪ Klason method for lignin ▪ Good mass balance closure ▪ Multiple samples considered	▪ Composition of extractives not determined ▪ Only one sample considered
3	▪ Data for both spring and fall harvests ▪ Good mass balance closure	▪ Forage fiber analysis used ▪ Acid detergent lignin analysis used ▪ Protein content not explicitly reported ▪ Only one sample considered
4	▪ Good mass balance closure	▪ Forage fiber analysis used ▪ Acid detergent lignin analysis used ▪ Only one sample considered
5	▪ Klason method for lignin	▪ Cellulose and hemicellulose content not quantified; only total carbohydrate ▪ Low mass balance closure (86.7%) ▪ Only one sample considered
6	▪ Klason method for lignin ▪ Good mass balance closure	▪ Cellulose and hemicellulose content not quantified; only total carbohydrate ▪ Protein not reported ▪ Switchgrass crop over-wintered; not representative ▪ Only one sample considered
7	▪ Multiple samples considered	▪ Forage fiber analysis used ▪ Acid detergent lignin analysis used ▪ Low mass balance closure (87.1%)
8	▪ Acid hydrolysis method for carbohydrates ▪ Klason method for lignin	▪ Protein not reported ▪ Low mass balance closure (88%)
9		▪ Analytical methods unknown
10a,b	▪ Data for both spring and fall harvests ▪ Multiple samples considered	▪ Forage fiber analysis used ▪ Permanganate lignin analysis used
11a,b	▪ Acid hydrolysis method for carbohydrates ▪ Klason method for lignin ▪ Good mass balance closure	▪ No data for spring harvest ▪ Only one sample considered

Table 4. Estimation of base-case switchgrass composition.

Component	(A) NREL Database Average Mass Fraction	(B) NREL Database Normalized Mass Fraction	(C) Extractives Composition	(D) Composition w/Extractives (B+C)	(E) Base-case Composition
Glucan	0.3330	0.3255	0.0239	0.3494	0.3427
Xylan	0.2220	0.2170		0.2170	0.2129
Arabinan	0.0290	0.0283	0.0007	0.0290	0.0285
Galactan	0.0100	0.0098	0.0057	0.0155	0.0152
Mannan	0.0030	0.0029		0.0029	0.0029
Total carbohydrate	(0.5970)	(0.5836)		(0.6138)	(0.6021)
Uronic acid	0.0140	0.0137		0.0137	0.0134
Lignin	0.1860	0.1818	0.0646	0.2464	0.2417
Protein	0.0490	0.0479	0.0009	0.0488	0.0478
Extractives	0.1160	0.1134			
Ash	0.0610	0.0596	0.0177	0.0773	0.0759
Acetate	n.r.				0.0190
TOTAL	1.0230	1.0000	0.1134	1.0000	1.0000

Table 5. Proposed base-case, first- and second-cut switchgrass compositions.

Component	Molecular Formula	LHV[8] (BTU/lb)	HHV (BTU/lb)	1st-cut Composition	2nd-cut Composition
Glucan	$C_6H_{10}O_5$	6,881	7,464[5]	0.3427	0.3427
Xylan	$C_50_8O_4$	7,072	7,645[5]	0.2129	0.2129
Arabinan	$C_50_8O_4$	7,072	7,645[5]	0.0285	0.0285
Galactan	$C_6H_{10}O_5$	6,881	7,464[5]	0.0152	0.0152
Mannan	$C_6H_{10}O_5$	6,881	7,464[5]	0.0029	0.0029
Total carbohydrate				(0.6021)	(0.6021)
Soluble solids[1]	$C_6H_{10}O_7$	5,279	5,766[6]	0.0134	0.0134
Lignin[2]	$C_{10}H_{13.9}O_{1.3}$	10,131	10,980[5]	0.1896	0.2417
Protein[3]	$C_1H_2O_{0.6}N_{0.3}S_{0.003}$	7,194	7,895[7]	0.1000	0.0478
Ash	See Table 6	0	0	0.0759	0.0759
Acetate	$C_2H_4O_2$	5,832	6,462[5]	0.0190	0.0190
TOTAL				1.0000	1.0000
LHV (BTU/lb)				7,011	7,164
HHV (BTU/lb)				7,609	7,770
Calculated Elemental Composition[4]					
C				0.4742	0.4914
H				0.0637	0.0646
O				0.3722	0.3615
N				0.0136	0.0065
S				0.0004	0.0002
Ash				0.0759	0.0759
TOTAL				1.0000	1.0000

[1]Soluble solids equivalent to uronic acid fraction from Table 4. Molecular formula is assumed to be equivalent to that for glucuronic acid.
[2]Lignin molecular formula is from NREL model (16).
[3]Protein molecular formula is derived from fresh alfalfa crude protein amino acid composition reported in Makoni et al., 1993 (17).
[4]Calculated from molecular formulae listed above.
[5]Value from NREL 2002 model.
[6]Value calculated as ∑product bond energies - ∑reactant bond energies for combustion of glucuronic acid.
[7]Value calculated from Schraer, W.D., H.J. Stoltze, 1999. *Biology: The Study of Life*. New York: Prentice Hall, pp. 151.
[8]LHV calculated as HHV – $[x_{H2}*x_{water}*H_{vap@25°C}]$ where x_{H2} = kg H_2/kg total; x_{water} = kg water/kg H_2; $H_{vap@25°C}$ = water heat of vaporization at 25°C.

Table 6. Ash composition (source: 7)

Component	Fraction of Ash	Normalized composition
Cl	0.0050	0.0050
SiO_2	0.5720	0.5693
Al_2O_3	0.0080	0.0080
Fe_2O_3	0.0037	0.0037
MgO	0.0480	0.0478
CaO	0.1110	0.1105
Na_2O	0.0030	0.0030
K_2O	0.0910	0.0906
P_2O_5	0.0550	0.0547
Lost on ignition	0.1080	0.1075
TOTAL	1.0047	1.0000

Table 7. Proposed advanced-case, first- and second-cut switchgrass compositions.

	Increased Carbohydrate[1]		Increased Lignin[2]	
Component	1st-cut Composition	2nd-cut Composition	1st-cut Composition	2nd-cut Composition
Glucan	0.3925	0.3925	0.3461	0.3432
Xylan	0.2438	0.2438	0.2150	0.2132
Arabinan	0.0326	0.0326	0.0287	0.0285
Galactan	0.0174	0.0174	0.0153	0.0152
Mannan	0.0033	0.0033	0.0029	0.0029
Total carbohydrate	(0.6895)	(0.6895)	(0.6081)	(0.6030)
Soluble solids	0.0102	0.0109	0.0136	0.0134
Lignin	0.1438	0.1964	0.2171	0.2768
Protein	0.1041	0.0498	0.1041	0.0498
Ash	0.0379	0.0379	0.0379	0.0379
Acetate	0.0144	0.0154	0.0192	0.0190
TOTAL	1.0000	1.0000	1.0000	1.0000
LHV (BTU/lb)	7,088	7,293	7,363	7,540
HHV (BTU/lb)	7,691	7,909	7,991	8,177
Calculated Elemental Composition				
C	0.4767	0.4939	0.5001	0.5198
H	0.0648	0.0656	0.0669	0.0679
O	0.4060	0.3955	0.3804	0.3673
N	0.0142	0.0068	0.0142	0.0068
S	0.0004	0.0002	0.0004	0.0002
Ash	0.0379	0.0379	0.0379	0.0379
TOTAL	1.0000	1.0000	1.0000	1.0000

[1]Advanced case high carbohydrate content = 1.1*base case carbohydrate content.
[2]Advanced case high lignin content = 1.1*base case lignin content.

PLEASE INDICATE WHETHER PROPOSED VALUES ARE APPROPRIATE; IF NOT, PLEASE RECOMMEND ALTERNATIVE

Table 8. Evaluation of proposed base-case and advanced-case compositions.

	Base Case				Advanced Case, Increased Carbohydrate				Advanced Case, Increased Lignin			
Component	1st-cut		2nd-cut		1st-cut		2nd-cut		1st-cut		2nd-cut	
Glucan	0.3427		0.3427		0.3925		0.3925		0.3461		0.3432	
Xylan	0.2129		0.2129		0.2438		0.2438		0.2150		0.2132	
Arabinan	0.0285		0.0285		0.0326		0.0326		0.0287		0.0285	
Galactan	0.0152		0.0152		0.0174		0.0174		0.0153		0.0152	
Mannan	0.0029		0.0029		0.0033		0.0033		0.0029		0.0029	
Total carbs	(0.6021)		(0.6021)		(0.6895)		(0.6895)		(0.6081)		(0.6030)	
Soluble solids	0.0134		0.0134		0.0102		0.0109		0.0136		0.0134	
Lignin	0.1896		0.2417		0.1438		0.1964		0.2171		0.2768	
Protein	0.1000		0.0478		0.1041		0.0498		0.1041		0.0498	
Ash	0.0759		0.0759		0.0379		0.0379		0.0379		0.0379	
Acetate	0.0190		0.0190		0.0144		0.0154		0.0192		0.0190	
TOTAL	1.0000		1.0000		1.0000		1.0000		1.0000		1.0000	
LHV (BTU/lb)	7,011		7,164		7,088		7,293		7,363		7,540	
HHV (BTU/lb)	7,609		7,770		7,691		7,909		7,991		8,177	
Elemental composition												
C	0.4742		0.4914		0.4767		0.4939		0.5001		0.5198	
H	0.0637		0.0646		0.0648		0.0656		0.0669		0.0679	
O	0.3722		0.3615		0.4060		0.3955		0.3804		0.3673	
N	0.0136		0.0065		0.0142		0.0068		0.0142		0.0068	
S	0.0004		0.0002		0.0004		0.0002		0.0004		0.0002	
Ash	0.0759		0.0759		0.0379		0.0379		0.0379		0.0379	
TOTAL	1.0000		1.0000		1.0000		1.0000		1.0000		1.0000	

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